

Cell sample storage for gene expression analysis on AmpliGrid AG480F

Besides cell deposition control and superior sensitivity an additional advantage of the AmpliGrid system is the reduced RNA degradation of cells stored on AmpliGrid slides. Reduced RNA degradation enhances data quality and consistency, particularly if workflows do not allow for PCR immediately to follow cell sorting. In this application report we determine RNA stability and integrity for a range of storage temperatures over a period of 5 weeks following cell deposition via flow cytometry.

Experimental Setup

Deposit HEK cells (human embryonic kidney cells) from a standard cell culture according to their physical parameters (forward and sideward scatter) on AmpliGrid AG480F slides using a Beckman Coulter MoFlo™ High Performance cell sorter. Store batches of AmpliGrid slides at room temperature, 4°C, -20°C or -80°C. Run 48 single cell RT-PCR reactions of the housekeeping gene Calmoduline (Calm) 0, 1, 2, 3, 4, and 5 weeks after deposition, using the Advantix One-Step RT-PCR kit. We use the PCR success rate as a proxy for the amount of RNA degradation at each time point and each storage temperature (the higher the PCR success rate, the less RNA degradation).

Cell sorting and deposition control

Sort single Hoechst stained HEK cells using a MoFlo™ High Performance cell sorter directly onto each of the 48 AmpliGrid reaction sites (fig. 1). Select vital cells based on their side and forward scatter signals.

Use nuclear staining with Hoechst dye to verify single cell deposition on the AmpliGrid reaction sites with a standard fluorescent microscope.

1 Figure 1: AmpliGrid AG480F slide and AmpliSpeed slide cycler ASC200D



One-Step RT-PCR

Pre-spot primer for Calm on the reaction sites by pipetting 1 µL of 0.6 µM primer (each sense and antisense) on the reaction sites and let them air dry.

Prepare the master mix for a one-step RT-PCR reaction on ice according to table A (Advantix Single Cell One-step RT-PCR kit).

A Table A: Single cell One-step RT-PCR master mix

Component	1 reaction	48 reactions
2x Single Cell RT Reaction Buffer	0.50 µL	30 µL
RNase Inhibitor (40 U/µL)	0.02 µL	1.2 µL
5x Single Cell RT Enhancer	0.15 µL	9 µL
Single Cell RT Enzyme Mix	0.04 µL	2.4 µL
Nuclease free water	0.29 µL	17.4 µL
Total volume	1 µL	60 µL

Pipette 1 µL of the master mix on each AmpliGrid reaction site and immediately cover with 5µL of sealing solution. Transfer the AmpliGrid to the AmpliSpeed slide cycler (preheated to 42°C) and start the amplification program as described in table B.

B Table B: One-step RT-PCR program

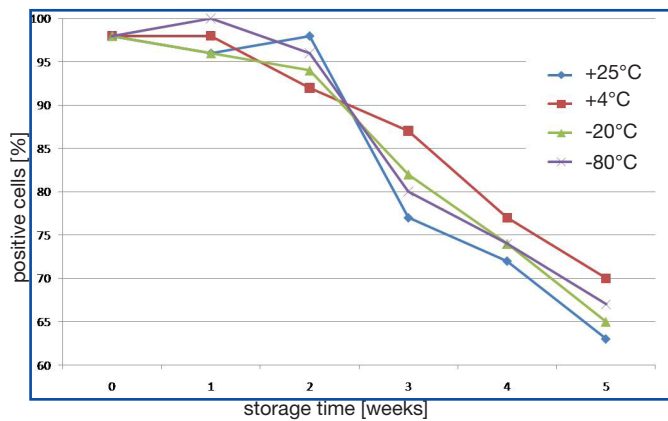
Temperature	Time	Cycle
42°C	10 min	
50°C	10 min	
58°C	30 min	
95°C	10 min	
94°C	30 sec	
60°C	75 sec	45
72°C	75 sec	
72°C	10 min	

After amplification apply 4 μL of 1.5x gel loading dye on the AmpliGrid reaction sites. Afterwards load samples directly onto a polyacrylamide gel and start the electrophoresis. Finally use a silver staining to visualize the bands on the polyacrylamide gel.

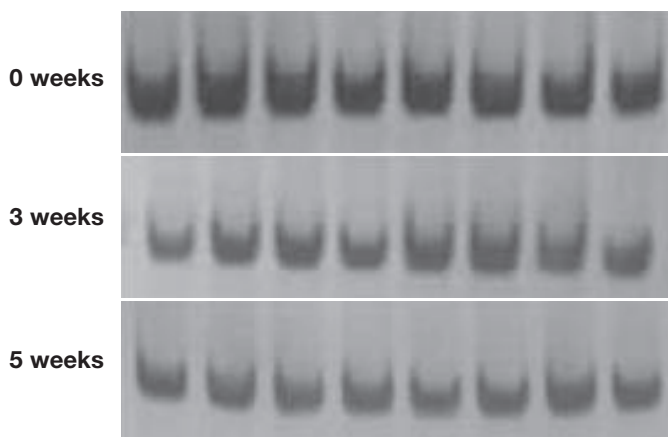
Results:

The results show very good preservation of RNA in the cells deposited on the AmpliGrid AG480F slide after 2 weeks of storage, even if stored at room temperature (see figure 2). Beyond 2 weeks, storage at 4°C yields the best results. The intensity of the bands slightly decreases over time (see figure 3), but bands are still visible even after 5 weeks and we achieved > 60% PCR success rate.

2 Figure 2: Graphical overview showing the percentage of positive reactions 0 - 5 weeks after flow sorting and a range of storage temperatures.



3 Figure 3: Typical gel image after 0, 3 and 5 weeks of storage at 4°C.



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Discussion:

We show that the dry storage of single cells on the AmpliGrid slides results in a very good preservation of cellular RNA which allows detection of the mRNA of a specific transcript even after 5 weeks of storage. We found best results for storage are temperatures of 4°C with minimal RNA degradation over a 2 week storage period. These results indicate that workflows that require a time lag between sorting and PCR processing are feasible with AmpliGrid. This is very important, e.g., in applications where samples are collected and sorted in various locations and PCR processing takes place in a central location.